

# Homotaurine: a GABA<sub>B</sub> antagonist in guinea-pig ileum

A. Giotti, S. Luzzi, S. Spagnesi & Lucilla Zilletti

Istituto Interfacoltà di Farmacologia e Tossicologia, Università di Firenze, Viale G.B. Morgagni 65, 50134 Firenze, Italy

- 1 Homotaurine (3-aminopropane sulphonic acid) did not inhibit the twitch response in guinea-pig ileum longitudinal muscle whilst  $\gamma$ -aminobutyric acid (GABA) and (–)-baclofen evoked dose-dependent inhibitions.
- 2 The inhibitory effects of GABA and (–)-baclofen were prevented in the presence of homotaurine  $2 \times 10^{-4}$  and  $10^{-3}$  M.
- 3 The log dose-effect curves of GABA and (–)-baclofen were shifted in a parallel manner compatible with competitive antagonism. The  $pA_2$  of homotaurine with GABA ( $4.22 \pm 0.05$ ) and (–)-baclofen ( $4.26 \pm 0.1$ ) were the same.
- 4 Homotaurine did not antagonize the inhibitory effects of morphine ( $ED_{50} 4 \times 10^{-7}$  M), noradrenaline ( $ED_{50} 10^{-6}$  M) or ATP ( $ED_{50} 1.5 \times 10^{-5}$  M).
- 5 The inferior homologue of homotaurine, taurine  $10^{-3}$  M, did not modify the inhibitory effects of GABA and (–)-baclofen.
- 6 Picrotoxin  $5 \times 10^{-5}$  M antagonized GABA<sub>A</sub> receptor-mediated contraction but did not affect GABA<sub>B</sub> receptor-mediated inhibition. At the same concentration the drug did not influence the antagonistic action of homotaurine, thus showing no GABA<sub>A</sub> receptor-mediated interference.
- 7 It may be concluded that homotaurine is a competitive antagonist of GABA<sub>B</sub> mediated effects in the guinea-pig ileum.

## Introduction

GABA ( $\gamma$ -aminobutyric acid) is a well-known neurotransmitter at the inhibitory synapses in the mammalian central nervous system (CNS) (Curtis & Johnston, 1974), where homotaurine (3-aminopropane sulphonic acid), the sulphonic analogue of GABA, acts as a potent and specific agonist of GABA receptors (Curtis, Phillis & Watkins, 1961; Enna & Snyder, 1975), while bicuculline is a specific antagonist (Curtis, Duggan, Felix & Johnston, 1971). The presence of GABA-'receptors' in the peripheral nervous system has been demonstrated by Bowery & Brown (1974), who observed a transient bicuculline-sensitive GABA-induced depolarization in rat superior cervical ganglion by means of recording with surface electrodes. In the myenteric plexus of the guinea-pig, the occurrence of GABA-neurones has been autoradiographically demonstrated by Jessen, Mirsky, Dennison & Burnstock (1979).

In non-stimulated preparations of guinea-pig isolated ileum, GABA evokes two kinds of response,

depending on the dose: relaxation, or contraction followed by relaxation (Giotti, Luzzi, Spagnesi & Zilletti, 1983). The classical GABA agonists, homotaurine and muscimol, cause contraction in the same preparations, while (–)-baclofen evokes relaxation (Kaplita, Waters & Trigg, 1982; Giotti *et al.*, 1983).

In supramaximally-stimulated preparations of guinea-pig isolated ileum, GABA-evoked contraction does not appear. GABA and (–)-baclofen inhibit only the twitch response, while homotaurine is devoid of inhibitory activity (Bowery, Doble, Hill, Hudson, Shaw, Turnbull & Warrington, 1981). It has been demonstrated by Kaplita *et al.* (1982) and by ourselves (Giotti *et al.*, 1983), that the contractions evoked by GABA, homotaurine and muscimol in non-stimulated preparations are mediated through classical bicuculline-sensitive receptors (GABA<sub>A</sub>-receptors). On the other hand, the relaxations and inhibitions caused by GABA and (–)-baclofen respectively in non-stimulated and supra-maximally-

stimulated preparations appear to be mediated through presynaptic bicuculline-insensitive GABA-receptors (GABA<sub>B</sub> receptors), as shown by Bowery *et al.* (1981) and also by ourselves (Giotti *et al.*, 1983).

GABA<sub>B</sub> receptors would appear to mediate pre-synaptic inhibition of neurotransmitter release in peripheral preparations such as guinea-pig and mouse vas deferens, rat atria (Bowery *et al.*, 1981), rat anococcygeus muscle (Muhyaddin, Roberts & Woodruff, 1982) and guinea-pig ileum (Bowery *et al.*, 1981; Giotti *et al.*, 1983). However, the significance of GABA<sub>B</sub> receptors in the peripheral nervous system has not been completely defined because of the lack of a specific antagonist.

The study described in the present paper was performed to ascertain whether homotaurine has any antagonist activity at peripheral GABA<sub>B</sub> receptors. The close similarity of the molecular structures of homotaurine and GABA prompted our investigation.

## Methods

Male guinea-pigs (weighing 300–500 g) were killed by a blow on the head; segments of terminal ileum were quickly removed and placed in a modified Krebs solution of the following composition (mM): KH<sub>2</sub>PO<sub>4</sub> 1.3, KCl 3.4, NaCl 134.7, CaCl<sub>2</sub> 2.8, MgSO<sub>4</sub> 0.6, NaHCO<sub>3</sub> 16.3, glucose 7.7.

Strips of ileal longitudinal muscle were obtained by the method of Paton & Zar (1968). Segments were mounted in an organ bath, bubbled with a mixture of 5% CO<sub>2</sub> and 95% O<sub>2</sub>, and maintained at 37°C. Field stimulation was performed following the method described by Paton (1963) and Paton & Vizi (1969). The stimuli (1.5 times maximal rectangular pulses of 1 ms duration, at a frequency of 6 per min) were applied using two coaxial platinum electrodes from a MARB stimulator. The ileum was connected to a MARB isometric transducer under a resting tension of 0.5 g and responses were recorded on a Battaglia-Rangoni FP 2400 polygraph. Preparations were allowed to equilibrate for 40 min before drug administration. Drugs were administered in a volume that never exceeded 1% of the total bath volume (4 ml). The occurrence of desensitization (Bowery *et al.*, 1981) was prevented by allowing 30 min to elapse between drug administrations. In fact, in preliminary experiments (*n* = 5) we observed that submaximal doses of GABA and (–)-baclofen, repeated at intervals ranging between 15–30 min, evoked the same inhibition of the twitch-response.

Dose-response curves were expressed as a percentage of the maximal effect elicited by GABA and (–)-baclofen. The drug antagonism was evaluated

following the pA<sub>2</sub> method reported by Van Rossum (1963). Linearization of experimental curves was performed following Clark (1926).

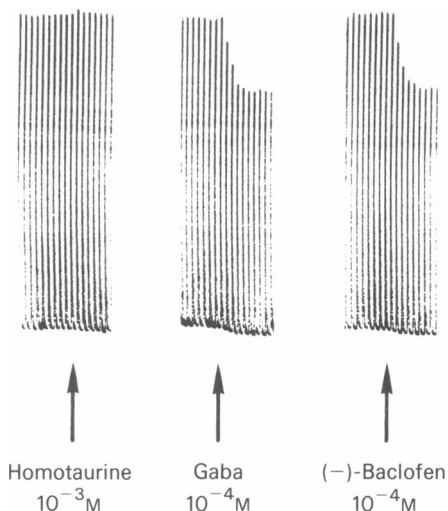
## Drugs

The following were used: γ-aminobutyric acid (GABA) (Sigma), 3-amino-1-propanesulphonic acid (homotaurine) (kindly supplied by Prof. G. Adembri), 2-amino-1-ethane-sulphonic acid (taurine) (kindly supplied by Prof. G. Adembri), (–)-β-(*p*-chlorophenyl)-γ-aminobutyric acid (–)-baclofen (kindly supplied by Dr W. Bencze, Ciba-Geigy), picrotoxin (Sigma), ATP (Boehringer, Mannheim), morphine hydrochloride (Sigma), noradrenaline bitartrate (Merck).

## Results

In electrically-stimulated ileal longitudinal muscle strips, GABA (*n* = 10) and (–)-baclofen (*n* = 12) caused a dose-dependent inhibition of contraction, while homotaurine at doses up to 10<sup>–3</sup> M caused no inhibition (Figure 1), as previously described (Bowery *et al.*, 1981).

To check for any interaction of homotaurine with



**Figure 1** Effects of homotaurine (10<sup>–3</sup> M), GABA (10<sup>–4</sup> M) and (–)-baclofen (10<sup>–4</sup> M) on the twitch response to field stimulation of the guinea-pig longitudinal muscle. The tissue was suspended under a resting tension of 0.5 g in a 4 ml organ bath containing a modified Krebs solution at 37°C. Stimuli (1.5 maximal rectangular pulses of 1 ms duration) were applied at a frequency of 6 min<sup>–1</sup>.

the GABA<sub>B</sub> receptor, dose-response curves for GABA ( $ED_{50} 9.3 \pm 1 \times 10^{-6} M$ ;  $n = 11$ ) and (-)-baclofen ( $ED_{50} 1.1 \pm 0.1 \times 10^{-5} M$ ;  $n = 16$ ) were determined before and after perfusing the preparation for 30 min with homotaurine. Homotaurine clearly antagonized the effects of GABA and (-)-baclofen on the twitch-response (Figure 2). After washing the preparation for 20 min with drug-free Krebs solution, an incomplete recovery was obtained, while after 30 min, responses had completely recovered (Figure 2). Homotaurine  $2 \times 10^{-4} M$  and  $10^{-3} M$  caused a parallel shift to the right in the dose-response curves for both GABA (Figure 3a) and (-)-baclofen (Figure 4a). In the presence of homotaurine  $2 \times 10^{-4} M$ , the maximal effects of GABA and (-)-baclofen were unchanged. After perfusion with homotaurine  $10^{-3} M$ , the maximal effects were not reached at the higher concentrations of GABA and (-)-baclofen used ( $10^{-3} M$ ). Concentrations above  $10^{-3} M$  were not tested.

Linearization of experimental curves with the Clark plot showed parallel displacement and demonstrated a competitive antagonism (Figures 3b and 4b).  $pA_2$  values of homotaurine for GABA and

(-)-baclofen were similar ( $pA_2$  for GABA  $4.22 \pm 0.05$ ;  $pA_2$  for (-)-baclofen  $4.26 \pm 0.1$ ). In other experiments homotaurine was added directly to the organ bath 15 s before adding GABA or (-)-baclofen. The effects of the drugs were not significantly different from those obtained when homotaurine was added to the perfusing medium 30 min before. Therefore homotaurine antagonism appears to be competitive and rapid in onset.

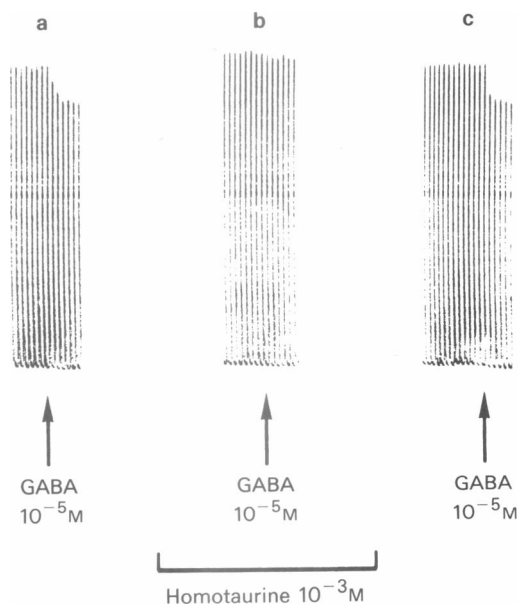
To check the specificity of the effect of homotaurine we studied its action on the inhibition of the twitch response caused by other substances acting through completely different receptor mechanisms; even at the higher doses ( $10^{-3} M$ ), homotaurine did not antagonize the effects of morphine ( $ED_{50} 4 \times 10^{-7} M$ ;  $n = 6$ ), ATP ( $ED_{50} 1.5 \times 10^{-5} M$ ;  $n = 5$ ) and noradrenaline ( $ED_{50} 10^{-6} M$ ;  $n = 5$ ).

The GABA<sub>B</sub> antagonist effect was restricted to homotaurine since the inferior homologue, taurine (2-amino-1-ethane-sulphonic acid), was devoid of any antagonist activity (Figures 5a and 5b) at the same concentrations.

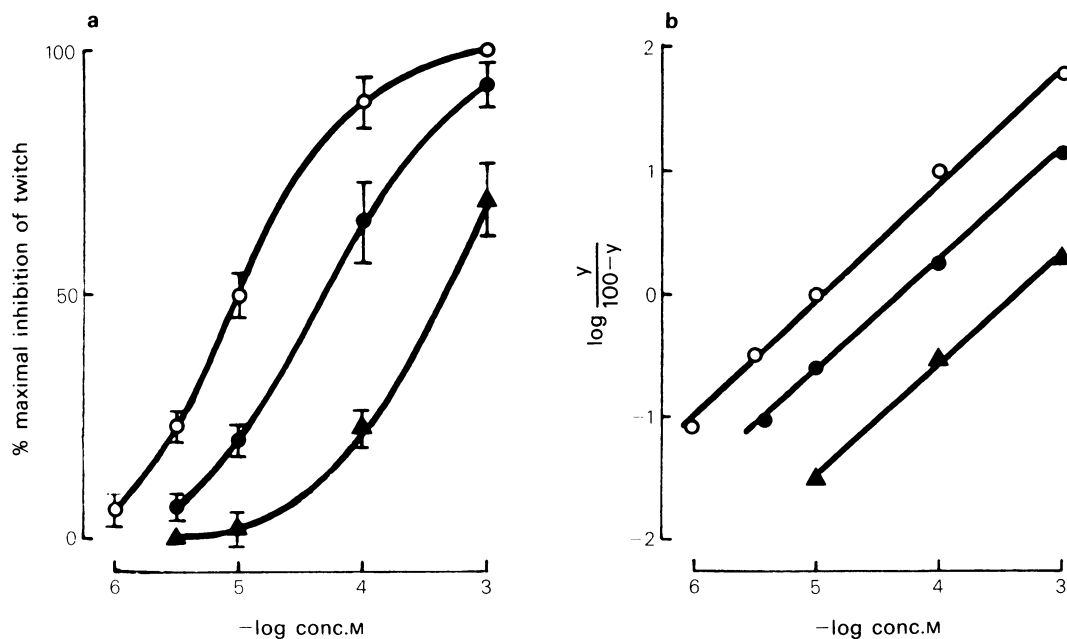
The possibility that the homotaurine effect might be due to its agonist action on the GABA<sub>A</sub> receptor (Kaplita *et al.*, 1982) was ruled out by using the GABA<sub>A</sub> antagonist, picrotoxin. The action of homotaurine ( $2 \times 10^{-4} M$  and  $10^{-3} M$ ) on GABA- and (-)-baclofen-induced inhibitions of the twitch response was studied in the absence and presence of picrotoxin ( $5 \times 10^{-5} M$ ) in the perfusing medium. Picrotoxin did not influence the antagonist action of homotaurine (Figure 6). At  $3 \times 10^{-5} M$  it is reported to antagonize the contractions mediated by GABA<sub>A</sub> receptors in non-stimulated preparations (Kaplita *et al.*, 1982).

In supramaximally-stimulated preparations the GABA- and homotaurine-evoked contractions did not appear (Figure 1), while they could be observed in submaximally-stimulated preparations, in which they were superimposed on the twitch response (Figure 7). These contractions were completely antagonized by picrotoxin  $5 \times 10^{-5} M$  (Figure 7). Moreover, picrotoxin does not influence the GABA<sub>B</sub>-mediated inhibition of the twitch response, as reported by Bowery *et al.* (1981) and also observed by us during this study (Figure 8). The GABA antagonist bicuculline was not used because in this preparation it increased the twitch-response by itself, probably due to its known anticholinesterase activity (Svenneby & Roberts, 1973).

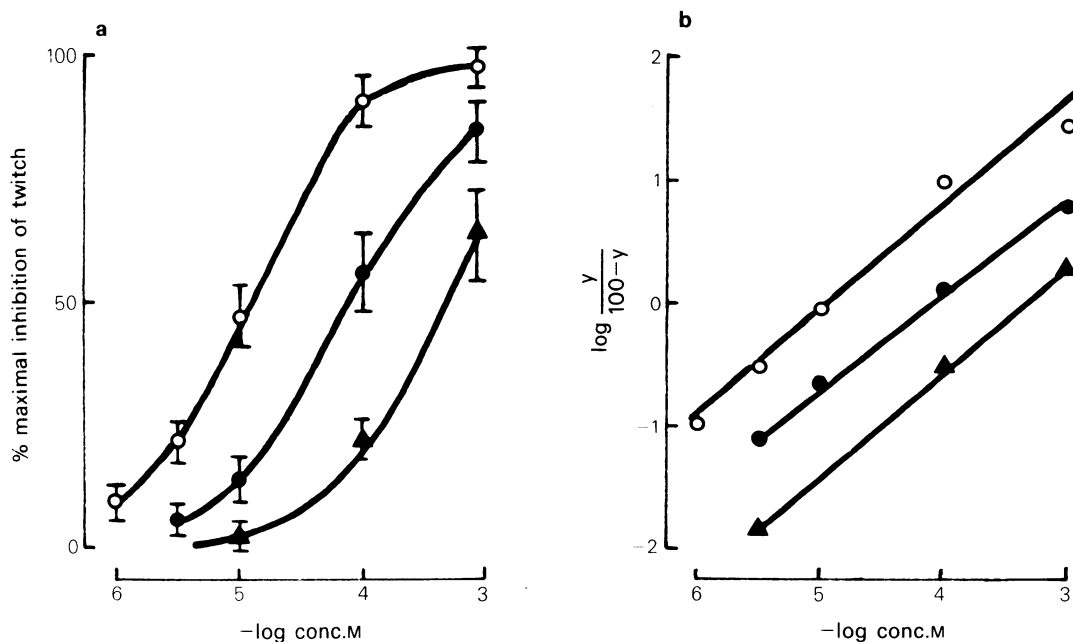
In a previous study (in some non-stimulated preparations: 10%) we observed a slight relaxant effect of homotaurine  $10^{-3} M$ , following its GABA<sub>A</sub> receptor-mediated contraction (Giotti *et al.*, 1983). The possibility therefore arises that the action of homotaurine described here could be a desensitization phenomenon rather than a competitive antagonism. The an-



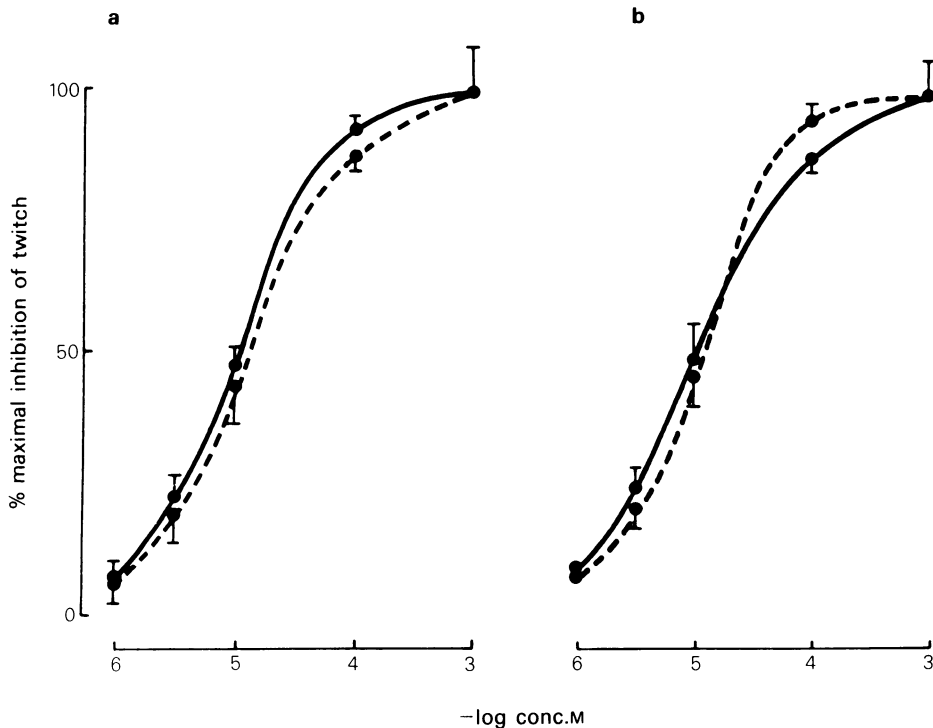
**Figure 2** (a) Effect of GABA  $10^{-5} M$  on the electrically-evoked response of the guinea-pig longitudinal muscle. (b) Inhibition of the effect of GABA  $10^{-5} M$  on the electrically-evoked twitch response of the guinea-pig longitudinal muscle in the presence of homotaurine  $10^{-3} M$  added to the bathing solution 30 min before GABA. (c) Recovery of the GABA effect after 30 min washing of the preparation.



**Figure 3** (a) Dose-response curves for GABA inhibition of the twitch response in guinea-pig ileum. Responses in normal perfusion solution (○); responses 30 min after addition to the bathing solution of homotaurine  $2 \times 10^{-4}$  M (●) and  $10^{-3}$  M (▲). Each symbol is the mean of at least 5 observations; vertical lines show s.e.mean. Effects are presented as a percentage of the maximal effect elicited by GABA; mean maximal inhibition was  $28 \pm 8\%$ . (b) Clark plot of homotaurine-induced antagonism on the GABA dose-response curve; symbols as in (a).



**Figure 4** (a) Dose-response curves of the (-)-baclofen inhibition of the twitch response in guinea-pig ileum. Responses in normal perfusion solution (○); responses 30 min after addition to the bathing solution of homotaurine  $2 \times 10^{-4}$  M (●) and  $10^{-3}$  M (▲). Each symbol is the mean of at least 5 observations; vertical lines show s.e.mean. Effects are presented as a percentage of the maximal effect elicited by (-)-baclofen; mean maximal inhibition was  $29.8 \pm 4.3\%$ . (b) Clark plot of homotaurine-induced antagonism on the GABA dose-response curve; symbols are the same as in (a).



**Figure 5** (a) Dose-response curves for the inhibition induced by GABA of the twitch response in guinea-pig ileum. Responses in normal perfusion solution (continuous line); responses 30 min after addition to the bathing solution of taurine  $10^{-3}$  M (dashed line). Each symbol is the mean of at least 5 observations; vertical lines show s.e. mean. Effects are presented as a percentage of the maximal effect elicited by GABA. (b) Dose-response curves for the inhibition induced by (-)-baclofen of the twitch response in guinea-pig ileum. Responses in normal perfusion solution (continuous line); responses 30 min after addition to the bathing solution of taurine  $10^{-3}$  M (dashed line). Each symbol is the mean of at least 5 observations; vertical lines show s.e. mean. Effects are presented as a percentage of the maximal effect elicited by (-)-baclofen.

tagonistic nature of this action was thus further studied in preparations perfused with picrotoxin  $5 \times 10^{-5}$  M, to avoid any GABA<sub>A</sub>-mediated effect. Homotaurine  $10^{-3}$  M, administered after GABA  $10^{-5}$  M had developed its maximal inhibitory effect, immediately reversed the GABA-evoked inhibition of the twitch response, while it did not influence the inhibitory action of morphine  $5 \times 10^{-8}$  M (Figure 9).

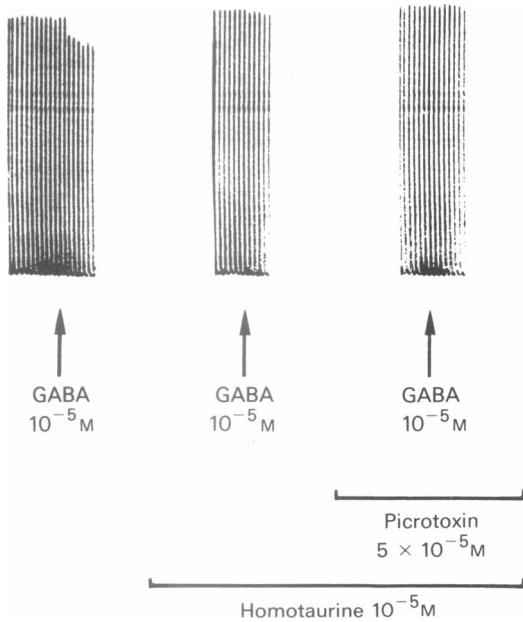
## Discussion

The experiments described in this paper concerning stimulated preparations of guinea-pig isolated ileum confirm that GABA and (-)-baclofen inhibit the twitch response through GABA<sub>B</sub> receptors (Bowery *et al.*, 1981). Our results, which are in agreement with those of Bowery *et al.*, also indicate that homotaurine exerts no agonist action on GABA<sub>B</sub> receptors in this preparation, and at the same time demonstrate that homotaurine antagonizes the GABA<sub>B</sub>-mediated ef-

fects of GABA and (-)-baclofen on the twitch response. This antagonism seems to be competitive in nature, since the dose-effect curves show a parallel displacement, and changes in the maximal effect are if anything small. However further experiments are necessary to substantiate this. It is interesting to note that  $pA_2$  values for GABA and (-)-baclofen are practically the same; this also suggests that homotaurine acts on a receptor site that is common to GABA and (-)-baclofen.

The effect is specific, since it is exerted only against GABA<sub>B</sub>-acting drugs, and not against those acting through a different receptor mechanism. The specificity is further proved by the fact that the inferior homologue of homotaurine, taurine, does not antagonize the GABA<sub>B</sub> receptor-mediated effect.

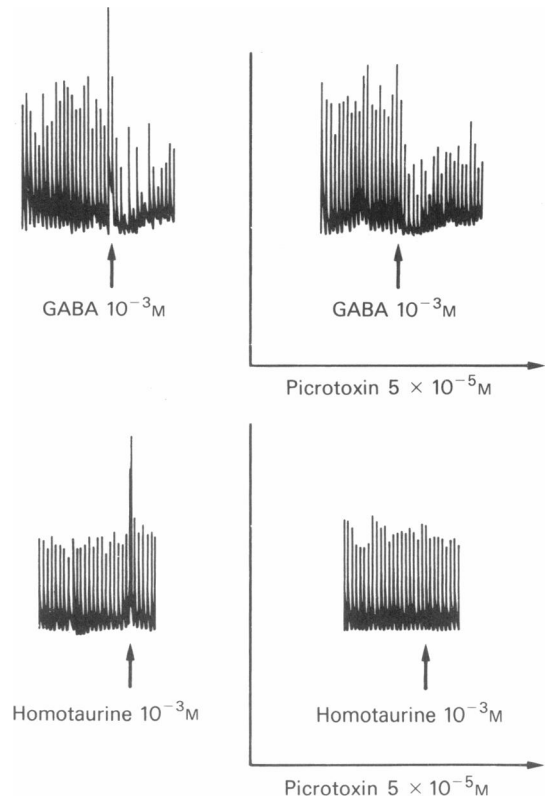
The potency of homotaurine would seem to be low in general terms, but it is of the same order as that of GABA and of (-)-baclofen as agonists. In fact, the  $ED_{50}$  for GABA and (-)-baclofen was approximate-



**Figure 6** Persistence of the antagonistic effect of homotaurine  $10^{-3}$  M on the relaxant effect of GABA  $10^{-5}$  M in the presence of picrotoxin  $5 \times 10^{-5}$  M.

ly  $10^{-5}$  M while the maximal effects was reached at doses of  $10^{-3}$  M.

Since a cross-desensitization has been demonstrated between GABA<sub>B</sub> agonists in peripheral tissue, it might have been postulated that the homotaurine effect was due to such a phenomenon. This was ruled out by the following facts: homotaurine by itself did not exert any GABA<sub>B</sub> agonist action at any of the concentrations tested in stimulated preparations. Although it caused a slight relaxation in 10% of non-stimulated preparations at  $10^{-3}$  M (Giotti *et al.*, 1983), which was probably related to some GABA<sub>B</sub> agonist activity, it must essentially be regarded as a GABA<sub>A</sub> agonist, possibly having a negligible GABA<sub>B</sub> agonist activity; the antagonism was demonstrated after a few seconds and it was clearly competitive. Moreover, homotaurine immediately reversed the action of GABA, but not that of morphine. All these findings taken together clearly point to a competitive antagonism and rule out desensitization. Homotaurine, however, is also a GABA<sub>A</sub> agonist in guinea-pig ileum, where it causes contraction (Kaplita *et al.*, 1982). Alternatively, the effect observed might thus be related to an excitation of GABA<sub>A</sub> receptors; we demonstrated, however, that the antagonistic effect of homotaurine was maintained when its GABA<sub>A</sub> effect was antagonized by

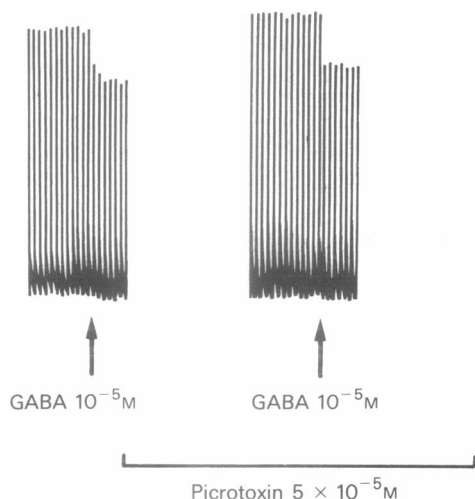


**Figure 7** Antagonism by picrotoxin  $5 \times 10^{-5}$  M of GABA  $10^{-3}$  M- and homotaurine  $10^{-3}$  M-evoked contractions in submaximally-stimulated preparation of guinea-pig ileum longitudinal muscle. Submaximal stimulation was performed by reducing the applied voltage to produce about half maximal twitch response. Picrotoxin  $5 \times 10^{-5}$  M was added to the perfusion solution 30 min before agonists were retested.

picrotoxin. Moreover, when the preparation was perfused for 30 min with homotaurine, GABA<sub>A</sub> receptors may be desensitized, as occurred in non-stimulated preparations (Kaplita *et al.*, 1982).

Our results thus indicate that the antagonism of homotaurine on the GABA<sub>B</sub> receptor-mediated effect is specific and receptor-mediated. Further evidence should arise from studies on peripheral GABA<sub>B</sub> binding.

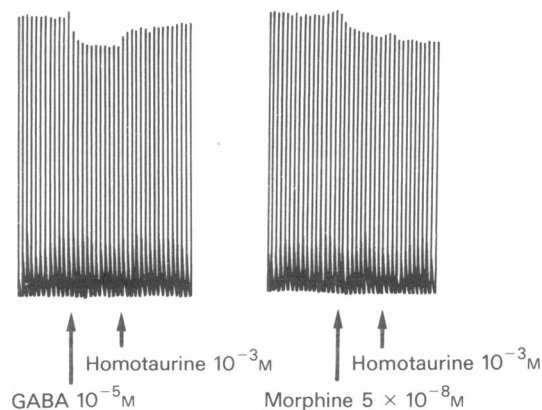
This result is interesting from two points of view; firstly, a structural analogue of GABA has been shown to possess antagonistic activity on GABA<sub>B</sub> receptors. This opens the way to a further investigation of different GABA analogues, with the aim of finding a more potent and specific GABA<sub>B</sub> receptor antagonist. During the preparation of this paper,



**Figure 8** Effects of GABA  $10^{-5}$  M on the twitch response to field stimulation of the guinea-pig longitudinal muscle in the absence and presence of picrotoxin  $5 \times 10^{-5}$  M added to the perfusion medium 30 min before the agonist was retested.

5-amino-valeric acid, the superior homologue of GABA, has been reported to antagonize the GABA<sub>B</sub>-mediated effect in rat anococcygeus muscle (Muhyaddin *et al.*, 1982), and the drug showed a lower potency than homotaurine in our stimulated preparations (unpublished observations).

Secondly, some hitherto unexplained differences between the central actions of GABA and homotaurine could be explained by the antagonistic GABA<sub>B</sub> activity of homotaurine. In fact homotaurine, as we have shown (Sgaragli, Carlà, Magnani & Giotti, 1978), has a desynchronizing



**Figure 9** Selective antagonism of homotaurine on GABA  $10^{-5}$  M- and morphine  $5 \times 10^{-8}$  M-induced inhibition of twitch response to field stimulation in the guinea-pig, isolated ileal longitudinal muscle. Homotaurine was added to the organ bath without washing when the inhibitory effect of GABA and morphine had developed.

effect on rabbit electrocorticograms, while GABA, by contrast, has a synchronizing effect. It has moreover recently been demonstrated (Fariello & Golden, 1980) that homotaurine potentiates epileptiform discharges in a feline model of corticoreticular epilepsy of the Petit Mal type, while GABA has no effect.

This work was supported by C.N.R. grant n. 81.0013704. The technical assistance of Marigrizia D'Asta is gratefully acknowledged. Correspondence to S.L. please.

## References

- BOWERY, N.G. & BROWN, D.A. (1974). Depolarizing action of  $\gamma$ -aminobutyric acid and related compounds on rat superior cervical ganglia *in vitro*. *Br. J. Pharmac.*, **20**, 205–218.
- BOWERY, N.G., DOBLE, A., HILL, D.R., HUDSON, A.L., SHAW, J.S., TURNBULL, M.G. & WARRINGTON, R. (1981). Bicuculline-insensitive GABA receptors on peripheral autonomic nerve terminals. *Eur. J. Pharmac.*, **71**, 53–70.
- CLARK, A.J. (1926). The antagonism of acetylcholine by atropine. *J. Physiol.*, **61**, 547–556.
- CURTIS, D.R., DUGGAN, A.W., FELIX, D. & JOHNSTON, G.A.R. (1971). Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord. *Brain Res.*, **32**, 69–96.
- CURTIS, D.R. & JOHNSTON, G.A.R. (1974). Amino acid transmitters in the mammalian central nervous system. *Ergebn. Physiol.*, **69**, 97–118.
- CURTIS, D.R., PHILLIS, J.W. & WATKINS, J.C. (1961). Actions of amino-acids on the isolated hemisectioned spinal cord of the toad. *Br. J. Pharmac.*, **16**, 262–283.
- ENNA, S.J. & SNYDER, S.H. (1975). Properties of  $\gamma$ -aminobutyric acid (GABA) receptor binding in rat brain synaptic membrane fractions. *Brain Res.*, **100**, 81–97.
- FARIELLO, R.G. & GOLDEN, G.T. (1980). Homotaurine: a GABA agonist with anticonvulsant effects. *Brain Res. Bull.*, **5**, 691–699.
- GIOTTI, A., LUZZI, S., SPAGNESI, S. & ZILLETTI, L. (1983). GABA<sub>A</sub> and GABA<sub>B</sub> receptor-mediate effects in guinea-pig ileum. *Br. J. Pharmac.*, **78**, 469–478.

- JESSEN, R.K., MIRSKY, R., DENNISON, M.E. & BURNSTOCK, G. (1979). GABA may be a neurotransmitter in the vertebrate peripheral nervous system. *Nature*, **281**, 71–74.
- KAPLITA, P.V., WATERS, D.M. & TRIGGLE, D.J. (1982).  $\gamma$ -Aminobutyric acid action in guinea-pig ileal myenteric plexus. *Eur. J. Pharmac.*, **79**, 43–51.
- MUHYADDIN, M., ROBERTS, P.J. & WOODRUFF, G.N. (1982). Presynaptic  $\gamma$ -aminobutyric acid receptors in the rat anococcygeus muscle and their antagonism by 5-aminovaleic acid. *Br. J. Pharmac.*, **77**, 163–168.
- PATON, W.D.M. (1963). Cholinergic transmission and acetylcholine output. *Can. J. Biochem. Physiol.*, **41**, 2637–2653.
- PATON, W.D.M. & VIZI, E.S. (1969). The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strips. *Br. J. Pharmac.*, **35**, 10–28.
- PATON, W.D.M. & ZAR, M.A. (1968). The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. *J. Physiol.*, **194**, 13–33.
- SGARAGLI, G.P., CARLA', V., MAGNANI, M. & GIOTTI, A. (1978). Homotaurine and muscimol mimic taurine and GABA effects on muscle tone and temperature regulation. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **305**, 155–158.
- SVENNEBY, G. & ROBERTS, E. (1973). Bicuculline and N-methyl-bicuculline competitive inhibitors of brain acetylcholinesterase. *J. Neurochem.*, **21**, 1023–1026.
- VAN ROSSUM, J.M. (1963). Cumulative dose-response curves. II Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Archs Int. Pharmacodyn.*, **143**, 299–330.

(Received October 29, 1982.  
Revised March 29, 1983)